# Xenoestrogens from Household Plastics Bind Estrogen Receptors and Affect Cell Proliferation

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Xenoestrogens are "foreign" chemicals or compounds that interact with estrogen receptors as either agonists or antagonists to interfere with endocrine system function. Xenoestrogens include phytoestrogens, pesticides, industrial by-products and synthetic estrogens. In this study, consumer plasticware, including baby bottles and food storage containers, were found to release xenoestrogenic compounds that bound to ERα and ERβ and affected cell proliferation.

## Introduction

Environmental endocrine-disrupting chemicals (EDC) affect cellular function by targeting receptors, drug metabolizing enzymes and transcriptional and translational machinery [1-3]. Xenoestrogens, a type of EDC, include phytoestrogens, mycoestrogens, pesticides, industrial by-products and synthetic estrogens [1-3]. These compounds may directly or indirectly affect estrogen receptor alpha and beta (ERα and ERβ) signaling [1-5]. Indirect mechanisms of activation or inactivation may involve coregulators or components of signal transduction cascades that crosstalk with estrogen receptor pathways [4-6]. Estrogen signaling pathways play an important role in reproduction, bone formation and maintenance, and cerebrovascular function, therefore identifying sources of EDC exposure is important to understanding epidemiological trends in endocrine-related diseases [1-4]. Several xenoestrogenic compounds have been identified in plastics manufacturing, including bisphenol A (BPA), phthalates, biphenyls and several alkylphenols [1-4]. In this study, receptor- and cell-based screening methods were used to determine if microwaving plastic food and laboratory containers, conditions typical of everyday use, causes the release of xenoestrogens.

## Methods

Polyethylene (PE), polypropylene (PP), polystyrene (PS), and polyvinylchloride (PVC) plastic containers were purchased from local retailers in South Carolina (baby bottles and foods storage containers). Glass Quorpak bottles and all other supplies were obtained from VWR (West Chester, PA). The containers were grouped into non-microwaved (NM) and microwaved (MW) treatments (see Figure 1). Deionized H2O (dH2O) was placed into each container to cover approximately ¼ of the surface area of each container. NM containers were the negative control. MW containers were microwaved at a maximum power (1200 watts) for 90 sec every other day for 30 days. Every 6 days, the water was transferred into glass containers and fresh dH2O water was placed into each MW container. After 30 days, pooled water samples from each container were extracted for xenoestrogens using C18 column chromatography and then run in the ERα and ERβ binding assays and a MTS cell proliferation assay [2]. For the MTS assay, a 96-well plate was seeded with 5000 T47D cells per well in RPMI media containing serum. 24 hours later, the media was replaced with RPMI media without serum. After 24 hours, water samples from the treatment groups were added to cell wells with 200 µl of RPMI without serum. The negative control was 1 µl DMSO and the positive control was 2 µl of 1 x 10-8 M estradiol-17β ($E_2$). After 48 hours, the media was removed and

Figure 1. Photo of containers. Container 1- PE storage container; 2- PP storage container; 3- PP food storage container; 4- unknown baby bottle; 5- unknown baby bottle; 6- PC baby bottle; 7- unknown baby bottle liner; 8- PE food storage bag; 9- PP food storage container; 10- polyethylene dish; 11- polyethylene dish; 12- PE drink container.

100 µl of RPMI was added to each well plus 25 µl of MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfo-phenyl)-H-tetrazolium). Plates were incubated at 37° C for 4 hr and read on a dual-wavelength spectrophotometer at 490 nm and 655 nm to assess cell proliferation. The data were analyzed by ANOVA using SAS (Cary, NC).

## Results

Results from receptor binding and cell proliferation assays are presented in Table 1 and Figure 2. In the ERβ assay, seven MW and three NM containers leached xenoestrogens. The three NM containers that leached xenoestrogenic chemicals were a plastic baby bottle (container 4), a PE bag (container 8) and a PE food storage dish (container 9). Leachate from containers 8 and 9 in both NM and MW groups displayed estrogen receptor binding activity. Only one MW container (container 1) a PE food storage dish, had activity in the ERα assay and also had the greatest level of binding in the ERβ assay. None of the NM samples registered quantifiable activity in the ERα assay. A significant difference was found between individual containers as well as pools of all MW and NM groups (2.2 ng/ml vs. 1.0 ng/ml, $P < 0.05$, n=22). Results from the MTS assay indicate that xenoestrogenic compounds released from plastics after microwaving can increase breast cancer cell proliferation.

## Discussion

The results from ER binding assays indicated that xenoestrogenic compounds were released from some plastic containers and that ERβ displayed greater affinity for these xenoestrogens than ERα.

Table 1. ERα and ERβ Binding Assays and MTS Cell Proliferation Assay

| Container | ERα | | ERβ | | MTS |
|---|---|---|---|---|---|
| | MW | NM | MW | NM | (%) |
| 1 | 140 | 0 | 630 | 0 | 23 |
| 2 | 0 | 0 | 0 | 0 | 0 |
| 3 | 0 | 0 | 40 | 0 | 38 |
| 4 | 0 | 0 | 2 | 140 | 4 |
| 5 | 0 | 0 | 0 | 0 | 1 |
| 6 | 0 | 0 | 0 | 0 | 5 |
| 7 | 0 | 0 | 0 | 0 | 0 |
| 8 | 0 | 0 | 40 | 50 | 0 |
| 9 | 0 | 0 | 30 | 40 | 34 |
| 10 | 0 | 0 | 280 | 0 | 87 |
| 11 | 0 | 0 | 20 | 0 | 0 |
| 12 | 0 | 0 | 10 | 0 | 0 |
| **Controls** | | | | | |
| Glass | 0 | | 0 | | 0 |
| Negative | 0 | | 0 | | 0 |
| Positive | 1290 | | 2160 | | 100 |

Note: Results for ERα and ERβ are expressed as estrogen binding equivalents (EBE). EBE is determined by calculating the displacement of labeled $E_2$ by extracts and standards from a $E_2$ standard curve. The positive control in the receptor assays was 5 ng diethylstilbestrol. Results from the MTS cell proliferation assay are expressed as a percentage of the positive control ($1 \times 10^{-8}$ M $E_2$).

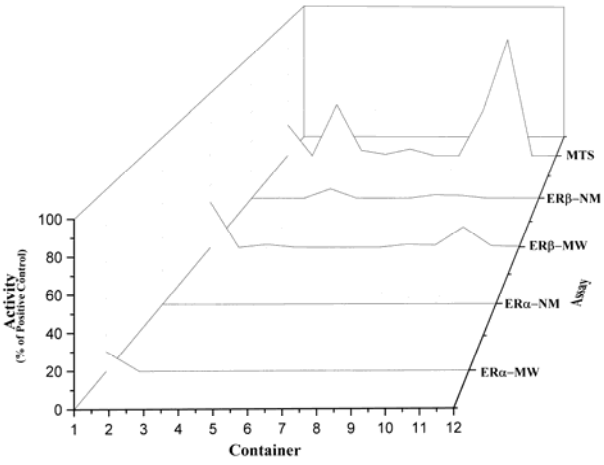


Figure 2. Results for microwaved (MW) and nonmicrowaved (NM) containers in MTS, ERα and ERβ receptor assays expressed as a percentage of estrogen positive control ($1 \times 10^{-8}$ M $E_2$).

Container 1 released chemicals capable of binding to ERα and ERβ and increasing breast cancer cell proliferation. Interestingly, some containers released xenoestrogenic compounds from both MW and NM groups; however, only MW samples increased breast cancer cell proliferation. A purple leachate was visible from containers 5 and 10 after column concentration. Container 10 had the highest amount of cell proliferation among plastic containers and it is not known whether the purple leachate interfered with quantification in the ER binding assays or stimulated cell proliferation through an ER-independent mechanism. In summary, some consumer plastics do release xenoestrogenic compounds that bind to both ERα and ERβ, display a greater affinity for ERβ than ERα and increase breast cancer cell proliferation. Further studies are warranted to investigate whether phthalate- and BPA-free products have reduced xenoestrogenic and cancer-stimulating activity [5].

## Notes and References

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